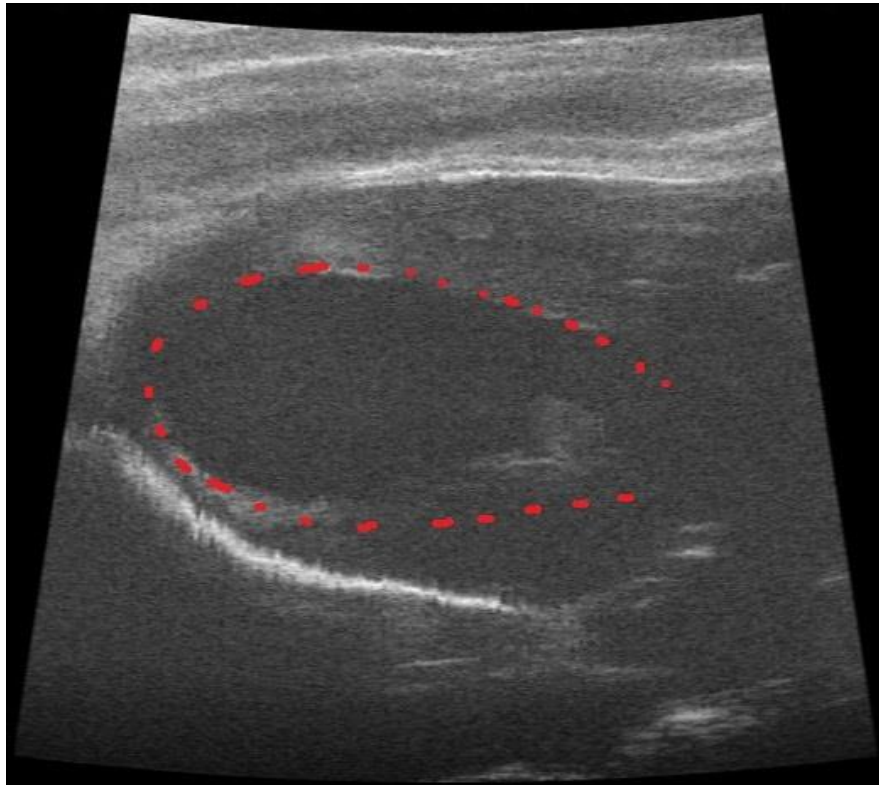


# Pathophysiology and treatment of Takotsubo Syndrome

## Insights from preclinical studies



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## **ABSTRACT**

**Background:** Takotsubo syndrome (TTS), also recognized as “stress-induced cardiomyopathy (SIC)” and “broken heart syndrome”, is an acute cardiac failure syndrome characterized by transient regional ventricular akinesia or hypokinesia, and often presents similarly to acute myocardial infarction (AMI). In contrast to AMI, the characteristic regional myocardial akinesia in TTS does not involve a culprit coronary artery. Although the associated cardiac dysfunction is potentially reversible, TTS is no longer regarded as benign. Today we know that TTS can lead to deleterious complications including death. Because physical and/or emotional stress often precedes TTS, acute sympathetic stimulation is understood to play a central role in pathophysiology of TTS, but the detailed mechanisms are still unclear. There are no clinical randomized studies in patients with TTS. Therefore, we currently lack guidelines for treatment.

**Aims:** The aims of this thesis were to study the reproducibility of our rat model, to unravel TTS pathophysiology and to explore possible prevention and treatment strategies in TTS. We aimed to investigate whether exogenous catecholamines can induce TTS-like cardiac dysfunction in rats.

**Methods:** We used 10-week-old Sprague Dawley rats in these preclinical studies. We based this work on our experimental TTS rat model which is based on exogenous isoprenaline. We tested whether different catecholamines, given intraperitoneally in titrated doses, could induce TTS. We studied possible preventive roles of  $\beta$ -blockers, ivabradine, sacubitril/valsartan and

cardiostimulants in our rat model. Blood pressure and heart rate were recorded via cannulation of right carotid artery. Small animal echocardiography was used to study cardiac morphology and function, and cardiac tissues were collected for histopathological studies.

**Results:** In **Study I**, we reproduced our isoprenaline-based rat model of TTS and showed that exogenous catecholamines can induce different patterns of TTS, depending on the associated blood pressure rather than their adrenergic receptor affinities. In **Study II**, we observed that phosphodiesterase inhibitor milrinone can induce typical TTS. We demonstrated that pretreatment with either  $\beta$ -non-selective antagonist or  $\beta$ 1-selective antagonist, but not  $\beta$ 2-selective antagonist, can prevent isoprenaline-induced TTS. Cardiostimulants failed to prevent isoprenaline-induced apical TTS. In fact, pretreatment with levosimendan worsened the degree of left ventricular apical akinesia. In **Study III**, we showed that ivabradine as well as heart block could prevent isoprenaline-induced TTS. Finally, in **Study IV**, we showed that pretreatment with sacubitril/valsartan can reduce mortality and prevent isoprenaline-induced TTS.

**Conclusion:** Our isoprenaline-based rat model of TTS is reproducible. Exogenous catecholamines can cause different variants of TTS depending on the associated haemodynamic profiles rather than catecholamine adrenergic receptor affinities. Hypotension and tachycardia precede isoprenaline-induced LV apical TTS in rats. Phenylephrine, ivabradine and  $\beta$ 1-selective antagonists could prevent typical TTS. These drugs mitigate the unique isoprenaline-associated haemodynamic profile. Takotsubo syndrome should, therefore, be regarded as an

acute sympathetic stimulation-induced *haemo-neuro-cardiac* syndrome rather than a cardiomyopathy per se. Inotrope and cardiostimulants such as milrinone and levosimendan can be deleterious in TTS.

**Key words:** Takotsubo syndrome, milrinone, levosimendan, echocardiography, isoprenaline, sacubitril/valsartan, ivabradine, stress-induced cardiomyopathy.



## **SAMMANFATTNING PÅ SVENSKA**

Takotsubo Syndrome (TTS) är akut hjärtsvikt sjukdom karakteriserad med akut potentiell reversibel myokardial akinesi, patienter med TTS presenteras oftast med symtom bild som liknar akut hjärt infarkt (AMI). TTS kan leda till allvarliga komplikationer som akut lungödem, synkope, arytmier, chock eller hjärtruptur. Mortalitet i TTS liknar detta i AMI. Patofysiologi i TTS är oklar, men stresshormoner och sympatisk stimulation tros spela stor roll. Det finns fortfarande inga randomiserade kliniska studier om TTS, varför vi saknar guidelines för behandling av patienter med TTS. Syftet med mitt doktorandprojekt är att studera om vi kan inducera TTS genom injicering av råttor med stresshormoner, kallas för catecholaminer. Vi syftade att studera patofysiologi av TTS från hemodynamisk effekter av catecholaminer på råttor och att utvärdera olika behandlingsalternativ. I **Study I**, visade vi att vi kan reproducera vår TTS råttamodell där isoprenaline injektion orsakar vänster kammaran apikalt akinesi. Vi visade också att olika catecholaminer kan leda till olika former av TTS baserat på associerat blodtryck. Manipulering av blodtryck medför omplacering av TTS akinesi position. **Study II**, visade vi att  $\beta$ -blockerande kan förebygga mot TTS i råttor. Hjärtstimulerande läkemedel, milrinone, kan orsaka TTS, precis som isoprenaline. Vi visade också att hjärtstimulerande mediciner försämrar isoprenaline inducerad TTS. I **Study III** och **IV**, vi visade pretreatment med ivabradine eller sacubitril/valsartan kan skydda mot utveckling av isoprenaline inducerad TTS i råttor.

Sammanfattningsvis har vi visat att catecholaminer kan leda till TTS av olika former,  $\beta$ -blockerande, ivabradine samt sacubitril/valsartan kan skydda mot TTS.





## LIST OF STUDIES

This thesis is based on the following studies, referred to in the text by their

Roman numerals (I–IV):

I: Redfors B, Ali A, Shao Y, Lundgren J, Omerovic E. **Different catecholamines induce different patterns of Takotsubo-like cardiac dysfunction in an apparently afterload dependent manner.** Int J Cardiol. 174 (2014) 330-336

II: Anwar Ali, Björn Redfors, Joel Lundgren, Jessica Alkhoury, Jonatan Oras, Li-Ming Gan, Omerovic E. **Effects of pretreatment with cardiostimulants and beta-blockers on isoprenaline-induced Takotsubo-like cardiac dysfunction in rats.** Int J Cardiol. 2019 Apr 15; 281:99-104

III: Anwar Ali, Björn Redfors, Joel Lundgren, Jessica Alkhoury, Jonatan Oras, Li-Ming Gan, Omerovic E. **The role of heart rate in isoprenaline-induced takotsubo-like cardiac dysfunction in rats.** Europ J Heart Fail. 2020 July 20.

IV: Anwar Ali, Björn Redfors, Jessica Alkhoury, Jonatan Oras, Li-Ming Gan, Omerovic E. **Sacubitril/valsartan decreases mortality and attenuates development of apical akinesia in rat model of isoprenaline-induced Takotsubo-like syndrome.** In manuscript.



# CONTENTS

ABBREVIATIONS	1
1. INTRODUCTION	3
1.1. Takotsubo syndrome	3
1.2. Epidemiology	4
1.3. Different morphological variants in TTS	4
1.4. Clinical presentation	5
1.5. Pathophysiology of TTS-current hypothesis	5
2. AIMS	6
3. METHODS	7
3.1. Animals	7
3.2. Vascular access and invasive haemodynamics recordings	7
3.3. Echocardiography	8
4. RESULTS AND CONCLUSIONS	8
4.1. Study I	8
4.2. Study II	9
4.3. Study III	10
4.4. Study IV	10
5. DISCUSSION	11
5.1. Takotsubo syndrome pathophysiology	11
5.2. Catecholamine-TTS relationship. The role of afterload in TTS pathophysiology	13
5.3. Our hypothesis	15
5.4. Our hypothesis revisited	17
5.5. The role of blood pressure in TTS pathophysiology	17
5.6. The role of heart rate in TTS pathophysiology	17
5.7. Sympathovagal imbalance and TTS	19
5.8. Typical TTS in absence of elevated catecholamines	20
6. TREATMENT OF PATIENTS WITH TTS	20
6.1. Prevention of TTS	20
6.2. Acute management of TTS	21
7. CONCLUDING REMARKS AND FUTURE PERSPECTIVES	22
8. ACKNOWLEDGEMENTS	23
9. REFERENCES	24
10. STUDY I	31
11. STUDY II	41
12. STUDY III	49
13. STUDY IV	61
14. APPENDIX	83



## **ABBREVIATIONS**

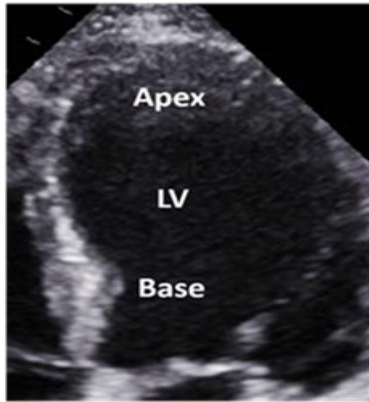
ACS	acute coronary syndrome
ADR	adrenaline
AMI	acute myocardial infarction
AVN	atrioventricular node
BP	blood pressure
BW	body weight
Cath	catheterization
CHB	complete heart block
DOP	dopamine
ECG	electrocardiogram
EKV	electrocardiogram-gated kilohertz visualization technique
ICI	selective $\beta_2$ antagonist
ISO	isoprenaline
IVAB	ivabradine
LA	left atrium
LEVO	levosimendan
LV	left ventricular/left ventricle
LVAW	left ventricular anterior wall
LVPW	left ventricular posterior wall
MET	metoprolol
MIL	milrinone
NOR	noradrenaline
PHE	phenylephrine
PROP	propranolol
SAC/VAL	sacubitril/valsartan
SAN	sinoatrial node
SIC	stress-induced cardiomyopathy

ST	sympathetic tone
TTS	Takotsubo syndrome
VAL	valsartan
VC	vasoconstriction

# 1. INTRODUCTION

## 1.1 Takotsubo syndrome

Takotsubo syndrome (TTS) is an increasingly recognized acute cardiac failure



**Fig.1** Left ventricle (LV) in 4 chambers view showing typical apical TTS

syndrome with a clinical presentation similar to that of acute myocardial infarction (AMI). Takotsubo syndrome is characterized by regional akinesia or hypokinesia of the myocardium, which, unlike in AMI, does not involve a culprit coronary artery.

The syndrome is also known as “broken heart syndrome” or “stress-induced cardiomyopathy (SIC)” as the majority of TTS patients have preceding somatic and/or emotional stress. Typical TTS presents with left ventricular<sup>1</sup> apical akinesia and basal hyperkinesia (**Fig. 1**). However, atypical TTS patterns such as basal<sup>2,3</sup>, global<sup>4</sup>, focal<sup>5</sup>, LV mid-ventricular<sup>2</sup>, biventricular<sup>6</sup> and even isolated right ventricle<sup>7</sup> involvement have been reported<sup>6</sup>.

Takotsubo syndrome is no longer regarded as benign as was once believed. We now know that it can lead to lethal complications such as malignant arrhythmias, thromboembolic events including stroke, cardiac rupture and cardiogenic shock<sup>8,9</sup>. A recent study has shown that mortality in TTS is equivalent to that in AMI<sup>10</sup>. Nevertheless, cardiac dysfunction in TTS is characteristically reversible if the patient survives the acute phase. Despite some proposals<sup>11-13</sup>, there is still no consensus on TTS-specific diagnostic criteria and management. Hence, preclinical studies utilizing a well-established TTS animal model are needed to pave the way

for a better understanding of the otherwise still ambiguous pathophysiology and for exploring different possible treatment options.

## **1.2 Epidemiology**

Takotsubo syndrome was first reported in six patients in 1985<sup>14</sup>. The first to describe the condition, in 1990, was the team of Dr. Hikaru Sato in Japan<sup>15</sup>. Since then, TTS has been increasingly reported worldwide<sup>11 16</sup>. Although elderly women are predominantly affected<sup>17</sup>, TTS can affect both genders of all ages<sup>18</sup>, even children<sup>19 20</sup> and neonates<sup>21</sup>. The condition accounts for 1–2% of acute coronary syndrome (ACS) patients<sup>22 23</sup>. The prevalence of TTS appears to be underestimated as, with increasing awareness, TTS was reported to increase by 20% in the period 2006–2012<sup>24</sup>. The reported TTS recurrence rate ranges from 0% to 22%<sup>25</sup>.

## **1.3 Morphological variants in Takotsubo syndrome**

Classically, typical TTS is characterized by extensive LV apical akinesia associated with LV basal hyperkinesia. Classically, atypical or reverse TTS is characterized by LV basal akinesia with preserved contraction at the LV apex. However, increasingly, different patterns of regional myocardial akinesia are being described<sup>23</sup>. Interestingly, the site of akinesia in TTS can change within the same admission<sup>26 27</sup> and in recurrent TTS<sup>27-34</sup>. It can also change in rats, based on the associated blood pressure (BP), as we showed in **Study I**.

In this thesis, the terms “typical” and “apical TTS” are interchangeably used to describe LV apical akinesia with a normally contracting base, while “atypical TTS” is used to refer to an akinetic LV base with a normally contracting apex.



## **1.4 Clinical presentation**

Takotsubo syndrome is an increasingly recognized acute cardiac syndrome. It is an important differential diagnosis in patients presenting with acute chest pain and acute heart failure. It often has a clinical picture similar to that of ACS. Typically, the patient is a postmenopausal woman presenting with acute anginal pain and/or dyspnoea. Preceding emotional and/ or physical stress may be present. Ischaemic electrocardiogram (ECG) changes, commonly pericordial T-wave inversion, are seen in most patients with TTS<sup>35 36</sup> and biomarkers of myocardial ischaemia are seen in virtually all TTS cases<sup>18</sup>. Takotsubo syndrome can be diagnosed by echocardiography. As AMI and TTS are not mutually exclusive and can co-exist<sup>37</sup>, distinguishing between AMI and TTS requires echocardiography/ventriculography and coronary angiography. The akinesia in TTS is often circumferential and associated with absence of explanatory coronary culprit lesions on the angiogram<sup>38</sup>. Physical stress, acute psychiatric or neurological disease, elevated troponin and low ejection fraction are independent risk factors associated with poor outcome in TTS<sup>17</sup>. In-hospital mortality has been reported to be 20.9% and 2.6% in TTS following physical and emotional stress, respectively.

## **1.5 Pathophysiology of Takotsubo syndrome – current hypothesis**

The pathophysiology of TTS is incompletely understood. Evidence suggests that sympathetic system stimulation plays a central role in TTS<sup>39</sup>. Patients with TTS have been shown to have elevated catecholamines<sup>40 41</sup>. Stress, sympathomimetic drugs<sup>42-45</sup> and endogenous as well as exogenous catecholamines<sup>46-49</sup> can all induce TTS.

Yet there is a knowledge gap regarding the causal relationship between sympathetic system stimulation and TTS<sup>50</sup>. The main postulations to elucidate this relationship are that excess adrenaline (ADR) changes the  $\beta$ 2 adrenergic receptor response from stimulation to inhibition<sup>51</sup>, causing preferential LV apical akinesia as there are more  $\beta$ 2 adrenergic receptors at the apex than in the rest of the LV; other postulations include coronary microvascular spasm; endothelial dysfunction; and local myocardial sympathetic stimulation<sup>52</sup>. These postulations are based on static anatomical distributions of myocardial vascular adrenergic nerves or  $\beta$ 2 adrenergic receptors. The widely accepted theory is based on the fact that the LV apex is rich in  $\beta$ 2 adrenergic receptors. Excessive ADR, by switching  $\beta$ 2 adrenergic receptor response from stimulatory to inhibitory, causes typical TTS.

However, the akinesia in TTS is neither confined to the LV nor LV apex. It is not necessarily static; in fact, it has been shown to be dynamic<sup>28-33</sup> and in **Study I**, we found that the site of LV akinesia in catecholamine-induced TTS depends on the associated BP rather than the adrenergic receptor affinity.

## **2. AIMS**

The general aims of this thesis were to unravel the pathophysiology behind TTS and to identify management options that could be beneficial for patients with TTS. The specific aims of each study in this thesis are given below:

**I:** To establish the catecholamine causation for TTS by studying whether our isoprenaline based TTS rat model is reproducible and whether other catecholamines can induce TTS in rats. We also aimed to explore the

pathophysiology of TTS inferred from effects of these catecholamines on haemodynamic profiles, lipid metabolism and cardiac function parameters.

**II:** To study the effects of pretreatment with cardiostimulants and  $\beta$ -blockers of different adrenergic receptor selectivity on development of LV apical akinesia in our ISO-based TTS rat model.

**III:** To explore whether pure heart rate reduction, using ivabradine (IVAB) or heart block, could prevent ISO-induced, TTS-like cardiac dysfunction in rats.

**IV:** To study the effects of pretreatment with sacubitril/valsartan (SAC/VAL) on development of ISO-induced TTS-like cardiac dysfunction in rats.

### **3. METHODS**

The methods used are detailed in the respective studies at the end of this thesis.

Below is a brief account of these methods:

#### **3.1 Animals**

In all studies, 10-week-old Sprague Dawley rats were used. In **Study I, II and IV**, male rats were used, while female rats were used in **Study III**. Experiments from our reproducible rat model of TTS, which is based on male rats, showed less data variance and, hence, strong statistical power. However, because the majority of TTS patients are females, we used female rats in **Study III**.

#### **3.2 Vascular access and invasive haemodynamic recording**

We dissected free and cannulated right internal jugular vein and right common carotid artery for delivery of parenteral infusion and continuous recording of BP, respectively. Small animal ECG systems were used to monitor heart rate. We used

different approaches, detailed in each study, to measure and analyse heart rate and BP.

### **3.3 Echocardiography**

We performed echocardiography on rats under general anaesthesia. Using ECG-gated technique (EKV), cine loops were acquired in long-axis view to check for development of TTS-like akinesia and cardiac dysfunction. The degree of TTS-like akinesia was traced along the endocardial border and expressed as a percentage of LV endocardial length. Echo images were optimized by consistent positioning of the ultrasound probe after maintaining the LV long-axis view showing the aortic valve, mitral valve and maximal LV lumen area.

## **4. RESULTS AND CONCLUSIONS**

Below are the main results of and conclusions drawn from each study. See the respective studies at the end of this thesis for detailed results and conclusions.

### **4.1 Study I**

**Different catecholamines induce different patterns of takotsubo-like cardiac dysfunction in an apparently afterload-dependent manner**

We have shown that catecholamines ISO, epinephrine, norepinephrine, phenylephrine <sup>54</sup> and dopamine (DOP) were all associated with development of SIC-like akinesia, or TTS-like cardiac dysfunction, in rats.

With the exception of ISO, all tested catecholamines caused increased BP and were associated with atypical, SIC-like dysfunction (LV basal akinesia with preserved apical contractility). Isoprenaline lowered the BP and was associated

with typical, SIC-like dysfunction (LV apical akinesia with preserved contractility at the LV base).

Lowering the BP to below 120 mmHg by arterial vasodilators in hypertensive catecholamine groups resulted in changing the TTS phenotype from basal to typical apical akinesia. By contrast, maintaining the BP at above 120 mmHg by PHE in the ISO group attenuated the TTS-like apical dysfunction and did not result in LV basal dysfunction.

We concluded that, in these catecholamine-based rat models of TTS-like cardiac dysfunction, low BP is associated with apical cardiac dysfunction while high BP is associated with LV basal cardiac dysfunction. We hypothesize that haemodynamic factors may be important in TTS pathogenesis and that TTS should be regarded as a cardio-circulatory syndrome rather than a mere cardiomyopathy.

## **4.2 Study II**

### **Effects of pretreatment with cardiostimulants and beta-blockers on isoprenaline-induced takotsubo-like cardiac dysfunction in rats**

We showed that milrinone (MIL) can induce typical apical LV akinesia in a manner similar to ISO. Pretreatment with either propranolol (PROP) or metoprolol (MET), but not with ICI 118551, attenuated ISO-induced, SIC-like cardiac dysfunction in a dose-dependent manner. Pretreatment with levosimendan (LEVO) accentuated apical ballooning, while pretreatment with MIL did not affect the degree of akinesia.

We concluded that Takotsubo-like cardiac dysfunction in rats can be induced by the phosphodiesterase inhibitor MIL. This conclusion challenges the notion that

catecholamine elevation precedes development of TTS. We provided experimental evidence for inotropes avoidance in managing patients with TTS.  $\beta_1$ , rather than  $\beta_2$ , adrenergic receptors may play a role in pathophysiology of TTS.

### **4.3 Study III**

#### **The role of heart rate in isoprenaline-induced takotsubo-like cardiac dysfunction in rats**

Similarly, induction of complete heart block (CHB) resulted in significant bradycardia and attenuated ISO-induced apical ballooning. We concluded that ISO-induced TTS-like cardiac dysfunction in rats can be prevented by mitigating ISO-induced tachycardia.

### **4.4 Study IV**

#### **Sacubitril/valsartan decreases mortality and attenuates development of apical akinesia in rat model of isoprenaline-induced takotsubo-like syndrome**

Compared with the control group, pretreatment with SAC/VAL resulted in less pronounced TTS-like cardiac dysfunction and lower mortality, while pretreatment with valsartan (VAL) alone did not achieve lowered cardiac dysfunction or mortality. Heart rate and BP were not significantly different between the groups.

We concluded that pretreatment with SAC/VAL, but not with VAL, reduces mortality and attenuates ISO-induced apical akinesia in the rat model of TTS.

Sacubitril/valsartan could be a potential treatment option in human patients with TTS.

## **5. DISCUSSION**

### **5.1 Takotsubo syndrome pathophysiology**

Despite numerous hypotheses, the precise pathophysiology of TTS is still unknown. Catecholamines as well as sympathomimetics can induce TTS<sup>42 46</sup> and patients with TTS have been reported to have high catecholamines levels<sup>55</sup> as well as sympathetic tone (ST)<sup>56</sup>. Therefore, sympathetic overstimulation and excess catecholamines are believed to play a causative role, but exact mechanisms remain speculative. The proposed mechanisms mainly include:

#### **Adrenaline- $\beta$ 2 adrenergic receptor switch theory**

Lyon et al hypothesize that excessive ADR, but not noradrenaline (NOR), causes  $\beta$ 2-rich LV apex akinesia by switching pathways: mediating first the stimulatory  $\beta$ 2-Gs protein pathway and then switching to the inhibitory  $\beta$ 2-Gi protein pathway<sup>51</sup>. This theory might explain typical TTS, but is difficult to reconcile with atypical TTS that spares LV apex. Of note is that other catecholamines that do not even act via  $\beta$ 2 can induce TTS<sup>46</sup>.

#### **Calcium overload theory**

Catecholamines at physiological levels increase cardiomyocyte-calcium cycling which enhances contractility. Calcium overload following release of excessive catecholamines that could jeopardize myocardial viability<sup>57</sup> has been suggested to explain the akinesia of TTS<sup>53</sup>.

### **Influx of inflammatory cells and free radicals**

Influx of inflammatory cells and free radicals into myocardium has been observed in TTS<sup>58 59</sup>. However, it is unclear whether such influxes have a role in TTS pathophysiology or merely follow tissue injury. Adrenergic overstimulation could cause myocardial injury that causes inflammatory cells influx.

### **Coronary macro/microvascular dysfunction**

Dote et al suggested coronary spasm to explain the occurrence of TTS after observing this lesion in two patients<sup>60</sup>; however, subsequent studies showed that the majority of TTS patients do not have coronary spasm<sup>61</sup>. The fact that dominant vasodilators, such as ADR and dobutamine, have been reported to cause TTS<sup>41</sup> argues against the coronary vasospasm hypothesis. Perfusion defects have been observed in patients with TTS. But these defects were reported in the aftermath of TTS<sup>62</sup>; therefore, it is not clear whether they are the cause or result of TTS. In our TTS rat model we reported normal perfusion at the akinesia sites<sup>63</sup>.

Although sympathetic stimulation plays a central role in TTS pathophysiology, there remain knowledge gaps regarding the catecholamine/sympathetic stimulation–TTS mechanistic relationship.

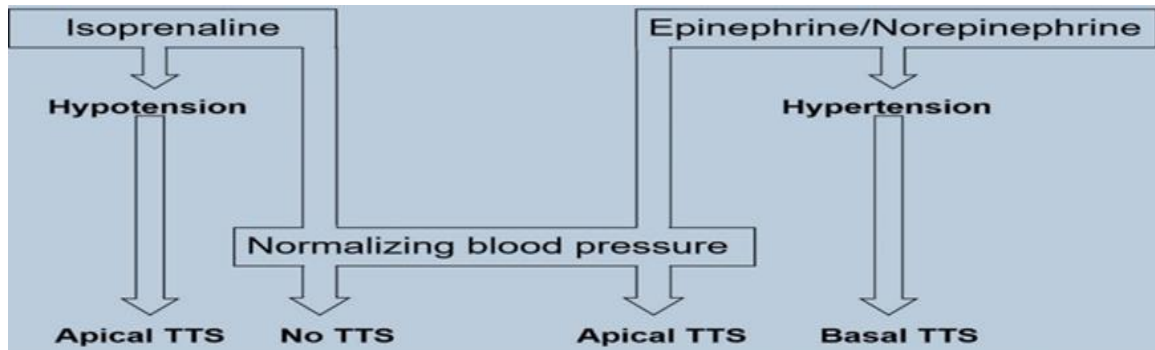
The findings of our experimental studies shed a light on the otherwise still unclear pathophysiology behind TTS<sup>64</sup>. There is a consensus that sympathetic stimulation plays a central role<sup>39</sup>. However, myocardial anatomical distribution of vessels, adrenergic receptors or nerves cannot explain the dynamic nature of TTS akinesia<sup>65</sup>.



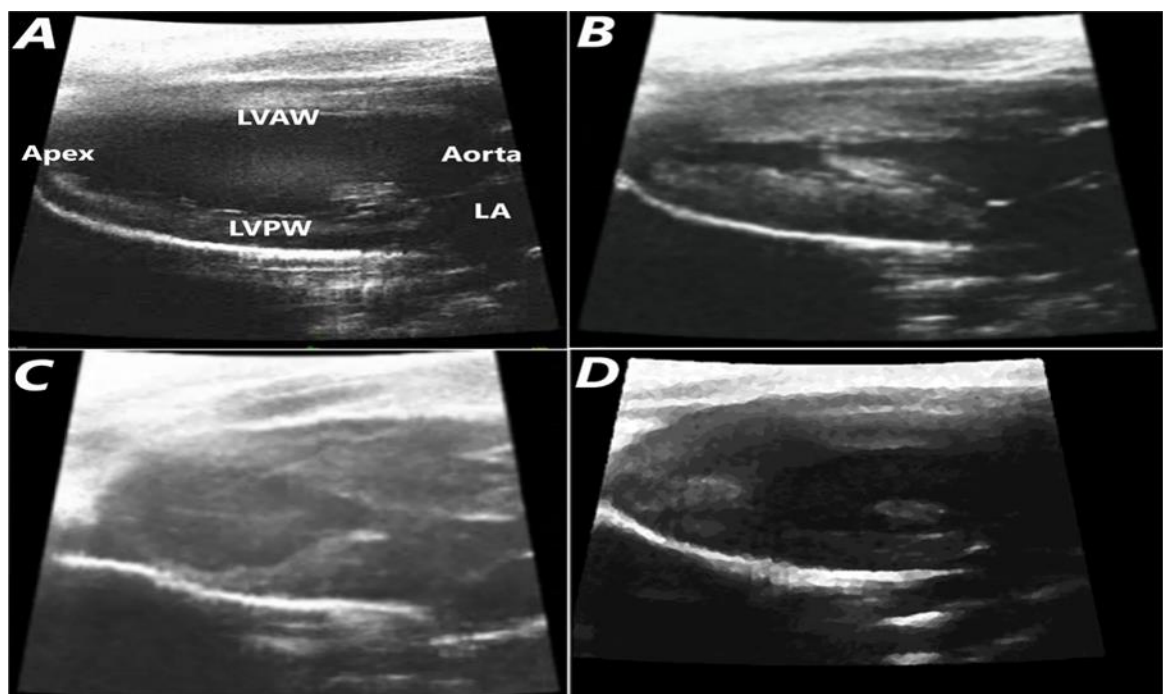
## 5.2 Catecholamine-Takotsubosyndrome (TTS) causal relationship. The role of afterload in TTS pathophysiology

In **Study I** we produced a rat model of TTS<sup>66</sup> where a single bolus dose of intraperitoneal ISO 50 mg/kg body weight (BW) produced most of the morphological and clinical picture of typical TTS in humans<sup>66</sup>. Importantly, aside from ISO, we showed PHE, ADR, DOP and NOR to induce atypical, LV basal TTS. Isoprenaline is a synthetic, non-selective  $\beta$ -adrenergic receptor agonist. In addition to its direct positive inotropic and chronotropic effects, it causes peripheral vasodilation, hence hypotension via  $\beta$ 2 adrenergic receptors<sup>67</sup>. By contrast, the other tested catecholamines induce vasoconstriction and hypertension via stimulation of their  $\alpha$ -adrenergic receptor<sup>68</sup>.

Contrary to ISO, which causes hypotension and a typical form of TTS, the other catecholamines fundamentally induce hypertension and atypical TTS. Intriguingly, the associated LV basal TTS pattern switched to typical, or apical, TTS with interventions to keep BP low. On the other hand, normalizing the otherwise low BP abolished ISO-induced apical TTS (**Fig. 2, Fig. 3**). Such dynamic nature of the akinesia site is reported in patients with TTS<sup>28-31 69 70</sup>.



**Fig. 2.** Isoprenaline, a pure  $\beta$ -adrenergic receptor agonist, induces hypotension and a typical, or apical, form of Takotsubo syndrome (TTS) that disappears when the otherwise low blood pressure (BP) is normalized. By contrast, epinephrine and norepinephrine induce hypertension and atypical basal TTS. Normalizing the BP results in changing the basal TTS to typical TTS.



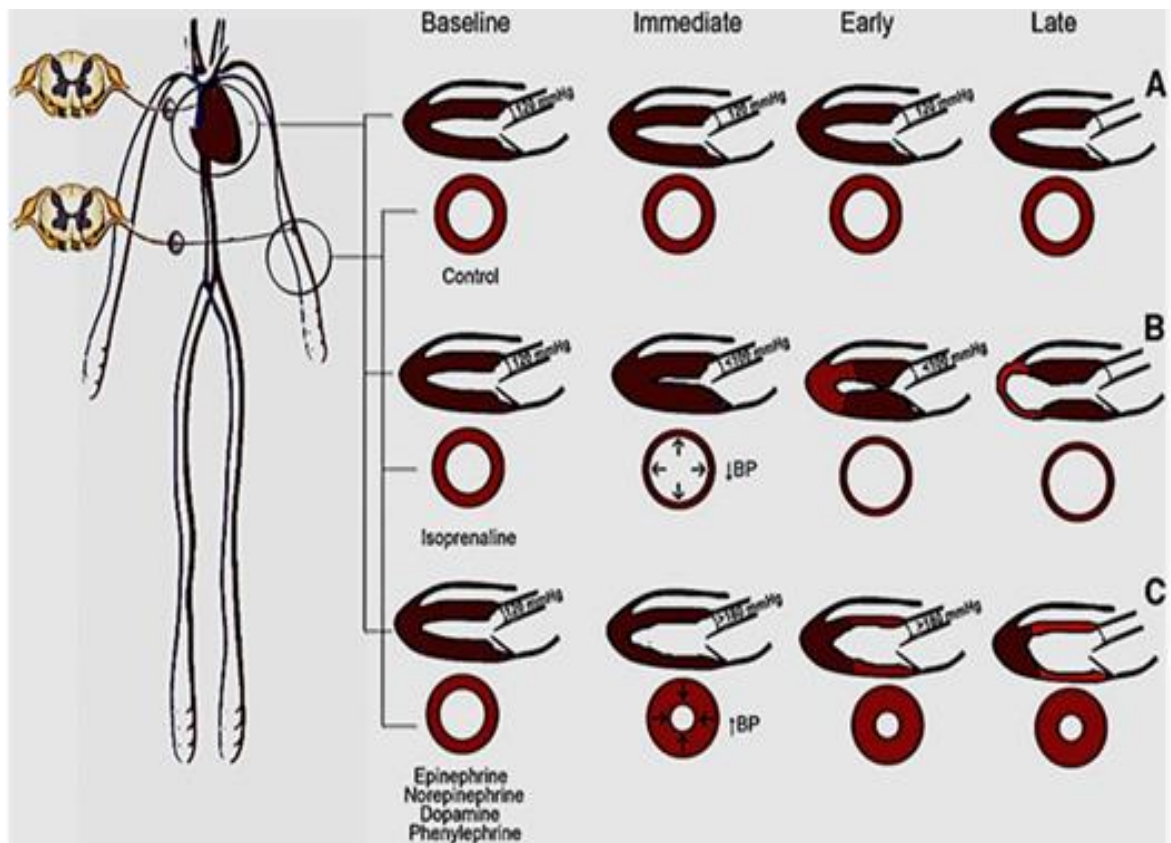
**Fig. 3.** Long-axis view of left ventricle. **A:** normal LV in diastole (LA = left atrium; LVAW = LV anterior wall; LVPW = LV posterior wall); **B:** normal LV in systole; **C:** typical, or apical, Takotsubo syndrome (TTS) in systole; and **D:** atypical basal TTS.

Based on our findings that exogenous catecholamines induced TTS, the form of which is dependent on whether the BP is high or low, we proposed this difference in BP to affect the distribution of LV regional wall tension that determines the TTS akinesia site.

In the context of exogenous catecholamines, hypotension induces intense inotropic drive that may cause near obliteration of LV outflow during systole and preferentially increases regional wall tension at the LV apex. On the other hand, hypertension mitigates the intense inotropic drive, and leads to less myocardial contraction and a different wall stress distribution pattern.

### **5.3 Our hypothesis**

We proposed that the regional mechanical overload plays a role in TTS pathophysiology (**Fig. 4**). Further, that cardiac cells in mechanically overloaded regions face excessive metabolic demands and a form of supply–demand mismatch. Takotsubo syndrome could essentially be protective against necrosis and akinetic cardiomyocytes could undergo protective metabolic shutdown for their survival. This may explain the high recovery rate seen in TTS.



**Fig. 4.** Proposal for catecholamines-TTS mechanistic relationship. Diagrams of the heart (in ends-systolic long axis view) and systemic arteries; (A): In a normal cardiac cycle, (B): Following isoprenaline injection & (C): Following administration of other catecholamines. A: The myocardium of left ventricle (LV) contracts uniformly during normal cardiac cycle. Appropriate cardio-arterial coupling is ensured by neural reflexes actions on vascular tone and cardiac contractility. B: Following isoprenaline, LV inotropy surges while LV afterload drops acutely due to vasodilation. Combined vasodilation & strong inotropy override neural reflex arcs. This increased contractility and reduced afterload leads to near obliteration of the lumen of LV lumen during systolic phase of cardiac cycle (immediate). After a while, apical akinesia develops (late). We hypothesize that transient LV outflow obstruction that develops when LV contracts too forcefully, generates a pressure gradient between the LV apex and the arterial system raising wall tension in regions located more apically than the obstruction (early). According to Laplace's law ( $T = P \times r/h$ ), combined high pressure and large volume at LV apex may jeopardize the function of apical cardiomyocytes. C: Following administration of the other catecholamines, blood pressure (BP) increases dramatically and LV basal akinesia with maintained LV apical contractility was noted. Once more, the robust sympathetic stimulation may override the neural reflex arcs. High peripheral resistance leads to global LV afterload. Here, LV in order to overcome this afterload, acquires more tension, thus ejection fraction decreases and outflow obstruction does not occur. Given that the heart is created to optimize blood dynamics, i.e. a flow of blood from LV apex to base, we hypothesize that when peripheral resistance is high, more early shortening occurs at LV apex while high afterload affects LV base

## 5.4 Our hypothesis revisited

Our hypothesis<sup>71</sup> is based on exogenous catecholamine-induced haemodynamic effects and the impacts of these effects on the distribution of myocardial wall tension.

## 5.5 The role of blood pressure in Takotsubo syndrome pathophysiology

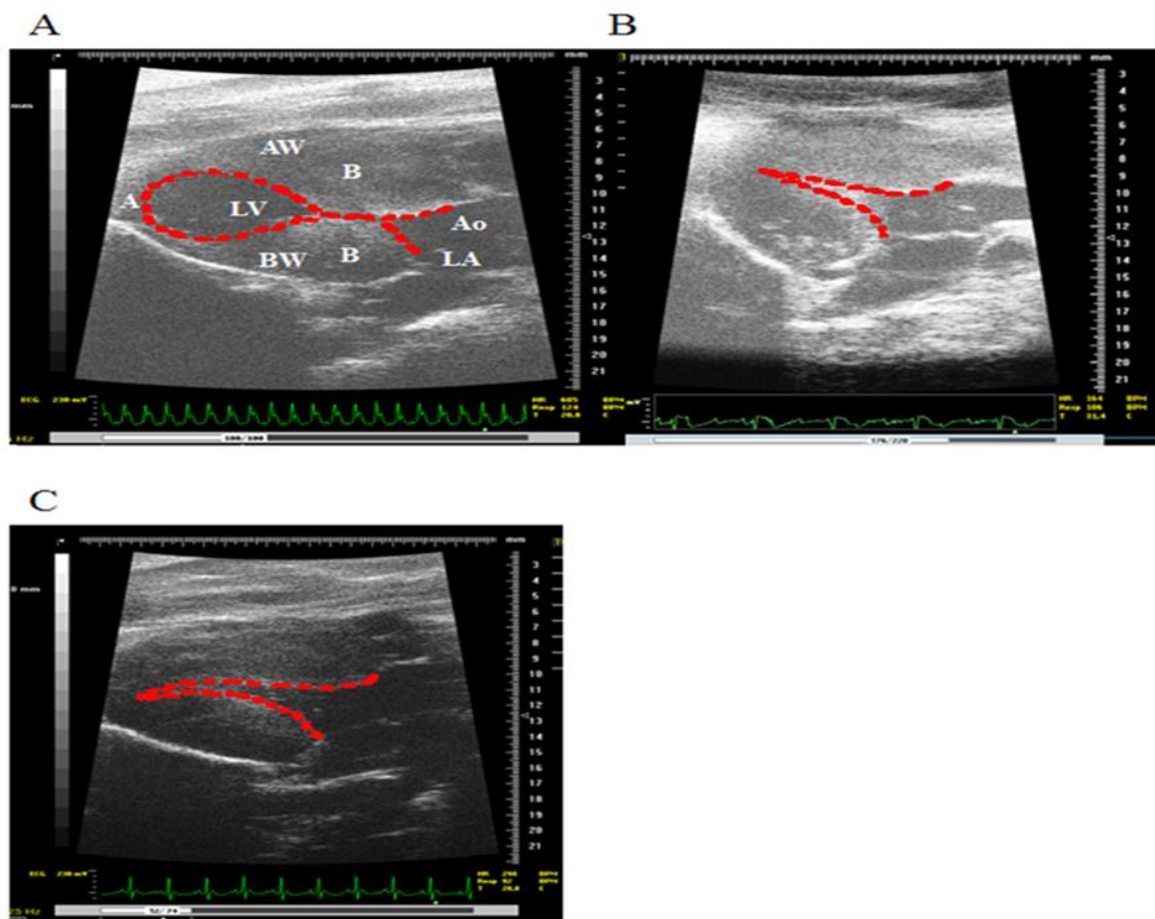
The pathophysiology of TTS could be multifactorial and complex<sup>72</sup>. But our consistent findings in **Study I**, that exogenous catecholamines induce typical LV apical or basal TTS depending on whether the associated BP is low or high, can be translated into haemodynamic-induced acute dysfunctional baroreflex input to the heart<sup>73</sup>. Catecholamines could cause TTS through an acute autonomic imbalance that interrupts normal sequential cardiac electromechanical synergy.

## 5.6 The role of heart rate in Takotsubo syndrome pathophysiology

In our rat model of TTS, the apparent LV outlet obstruction could be a result of ISO-induced unopposed sympathetic storm rather than a cause of LV apical akinesia. Normalizing BP, which could mean mitigating sympathetic overstimulation of cardiac conduction system, and, hence, less tachycardia, prevented ISO-induced apical TTS, as shown in **Study I**. Furthermore, in **Study II**, we found a strong positive association between heart rate and the extent of LV apical akinesia. We reported that  $\beta$ -blockers mitigate tachycardia and ISO-induced apical TTS. We also found that a high dose of LEVO exacerbated the ISO-induced TTS. Selective blockade of  $\beta_1$ , but not  $\beta_2$ , adrenoreceptors reduces the extent of akinesia. Together, these results indicate that excessive inotropic and chronotropic



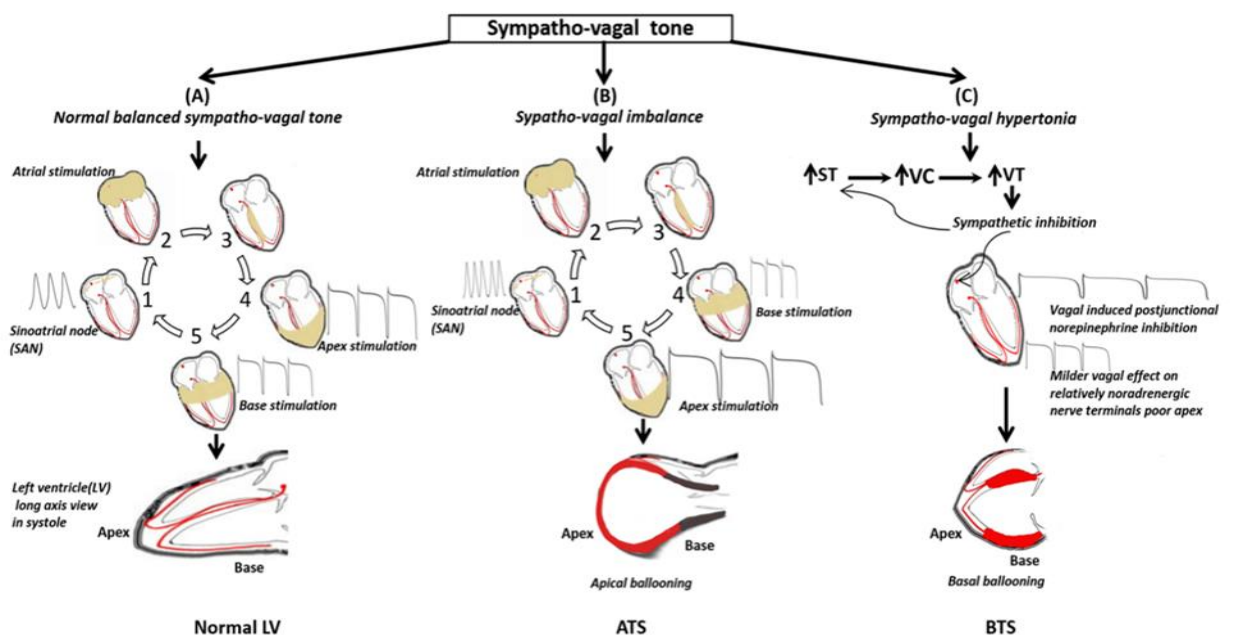
stimulation, and  $\beta_1$  rather than  $\beta_2$  adrenoreceptors, play an important role in the pathophysiology of TTS. The next step was to study whether non-beta blocker bradycardia could prevent ISO-induced apical TTS in rats. To obtain pure heart rate reduction, we used IVAB and mechanical heart block. Consequently, in **Study III** we found IVAB as well as heart block to prevent ISO-induced apical TTS (**Fig. 5**).



**Fig. 5.** Long-axis view of the left ventricle in systole. **A:** Isoprenaline <sup>53</sup> induced an apical Takotsubo syndrome (TTS) pattern (A = apex; Ao = aorta; AW = anterior wall; B = base of LV; BW = posterior wall; LA = left atrium). **B:** Heart block abolished ISO-induced TTS. **C:** Ivabradine (IVAB) prevented ISO-induced TTS.

## 5.7 Sympathovagal imbalance and Takotsubo syndrome

There is knowledge gap regarding what happens between acute sympathetic stimulation and development of different TTS variants. At the end of this thesis, we propose that sympathovagal interplay may have a role in TTS pathophysiology (**Fig. 6**). Briefly, vagal withdrawal induced by hypotension<sup>54 74</sup> and ISO<sup>75-77</sup> exposes the heart to unopposed, yet augmented sympathetic stimulation that could reverse the normal apex-to-base repolarization<sup>78, 79</sup> and prolong the action potential more at the apex than at the base of the LV<sup>79</sup>. This could explain the LV basal electromechanical dominance over the apex, with ISO producing the apical variant of TTS. Atypical basal TTS, as seen in **Study I**, could be related to catecholamine-induced exaggerated vagal response to sympathetic stimulus<sup>80 81</sup>. Future studies are needed to explore the role of electromechanical dyssynchrony in pathophysiology of TTS.



**Fig. 6. A:** During normal sinus rhythm, activation starts at the sinoatrial node (SAN) and progresses via the atrioventricular node (AVN) to stimulate the left ventricle from the apex to the base. **B:** This sequence is reversed with unopposed sympathetic overstimulation, resulting in LV basal electromechanical dominance over the apex, which, in turn, results in apical Takotsubo syndrome (TTS). **C:** Elevated sympathetic tone (ST) causes peripheral vasoconstriction (VC) that could in turn cause basal TTS via accentuated vagal hypertonia-induced LV proximal akinesia.

## **5.8 Typical Takotsubo syndrome in the absence of elevated catecholamines**

In **Study II**, we found that MIL, a phosphodiesterase inhibitor that does not act directly via adrenoceptors, induces typical TTS, similar to ISO, in rats. This finding suggests that TTS can occur in the absence of elevated catecholamines and it challenges the concept that high levels of circulating catecholamines or direct excessive stimulation of myocardial adrenergic receptors are necessary for the development of TTS. Like ISO<sup>75-77, 82 83</sup>, MIL accelerates cardiac conduction<sup>84-86</sup> and induces vasodilation<sup>86-89</sup>, which could cause vagal withdrawal<sup>54 74</sup>. Hypotension and tachycardia are commonly associated with apical TTS induced by ISO and MIL.

## **6. TREATMENT OF PATIENTS WITH TAKOTSUBO SYNDROME**

There have been no prospective randomized clinical trials in patients with TTS. Therefore, treatment guidelines for TTS are currently lacking. In TTS patients, beta-blockers mitigate the severity of recurrent TTS<sup>90 91</sup>. However, reports about treatment with beta-blockers are contradictory. Some recommended inclusion of beta-blockers in the TTS treatment protocol<sup>1</sup>, while others reported that beta-blockers have no benefit regarding short<sup>7</sup> and long-term mortality<sup>7</sup> in TTS.

### **6.1 Prevention of Takotsubo syndrome**

The role of beta-blockers in TTS management is therefore controversial<sup>91</sup>. Our findings in **Study II** indicate that cardio-selective beta-blockers could prevent apical TTS. Pretreatment with MET led to a dose-dependent reduction in TTS akinesia and mortality in rats. Similar effects of MET on TTS were reported in



monkeys<sup>92</sup>. Beta-blockers were reported to reduce severity in recurrent TTS<sup>93</sup>. We found MET 50 mg/kg BW to prevent ISO-induced apical TTS.

In **Study III**, we showed a beneficial role of pretreatment with IVAB in prevention of ISO-induced TTS in rats. These findings are in accordance with reports showing that IVAB plays a role in treatment of TTS patients<sup>94-96</sup>.

## **6.2 Acute management of Takotsubo syndrome**

Ideally, patients with TTS are managed at a cardiology unit with a catheterization<sup>17</sup> lab and imaging facilities. Currently, there are no randomized clinical trials (RCT) in TTS that we can base our recommendations on. First, do no harm (Primum nil nocere) should be recommended as TTS is largely regarded as self-healing syndrome.

Cautious use of beta-blockers seems reasonable<sup>97</sup> as catecholamines and sympathetic stimulation play a central role in TTS pathophysiology. In **Study II**, we showed that MET and PROP can prevent ISO-induced apical TTS in rats.

We also showed that a high dose of LEVO worsened ISO-induced TTS and that selective blockade of  $\beta_1$ , but not  $\beta_2$ , adrenoreceptors reduces the extent of akinesia. These results provide experimental evidence for avoidance of positive inotrope in treatment of TTS<sup>98</sup> and that  $\beta_1$ , rather than  $\beta_2$ , adrenoreceptors play an important role in the pathophysiology of TTS.

## 7. CONCLUDING REMARKS AND FUTURE PRESPECTIVES

We reproduced our ISO injection-based rat model that contains most morphological and clinical features of TTS. We provided experimental evidence for a catecholamine–TTS causation relationship by showing that TTS can be induced by exogenous catecholamines.

The site of akinesia in catecholamine-induced TTS is determined by afterload. In this context, low afterload leading to an unopposed sympathetic storm, and high afterload leading to acute augmented vagal hypertonia, both of which could interrupt normal LV electromechanical synchrony. LV base electrical dominance could precede its mechanical dominance over the apex in ISO-induced apical TTS.

Interruption of sympathetic overstimulation at the sinoatrial node (SAN) or atrioventricular node (AVN) by IVAB or CHB, as well as pretreatment with MET, prevented ISO-induced typical TTS in rats. These findings highlight the role of ISO-induced augmented unopposed overstimulation of the sympathetic cardiac conduction system in the pathophysiology of apical TTS. Future studies need to investigate whether LV electromechanical dyssynchrony precedes development of akinesia in TTS.

$\beta$ -blockers and IVAB could have a role in prevention and treatment of TTS. Randomized controlled studies are needed to establish TTS treatment guidelines.

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